



Optimization of Methods to Visualize Hydrogen Peroxide at Cellular Membranes

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Background

Membranes can be fluorescently tagged by treating cells with an azide-functionalized, clickable handle that is incorporated into membranes using an enzymatic pathway. Subsequent “click” chemistry with a functionalized fluorophore allows for visualization of membranes with confocal microscopy. We hypothesize that a pro-fluorescent compound known as an activity-based sensor (ABS) will be able to report the presence of hydrogen peroxide at cellular membranes.

Research Goals

- Prepare and purify reported peroxyfluor-1 (PF1) hydrogen peroxide ABS
- Prepare and purify of clickable analog
- Compare analyte selectivity of PF1 and the clickable analog using UV-Vis and fluorescence spectroscopy
- Utilize click chemistry to functionalize membranes with the pro-fluorescent reagent

Timeline of Progress

- ✓ Preparation of the control fluorophore PF1¹ to compare data
- ✓ Preparation of the pro-fluorescent ABS reagent with the carboxylic acid attached
- ✓ Purification of the PF1 analogue
- Compare fluorescence data of PF1 with the functionalized compound to ensure that analyte selectivity is maintained

Synthesis of PF1

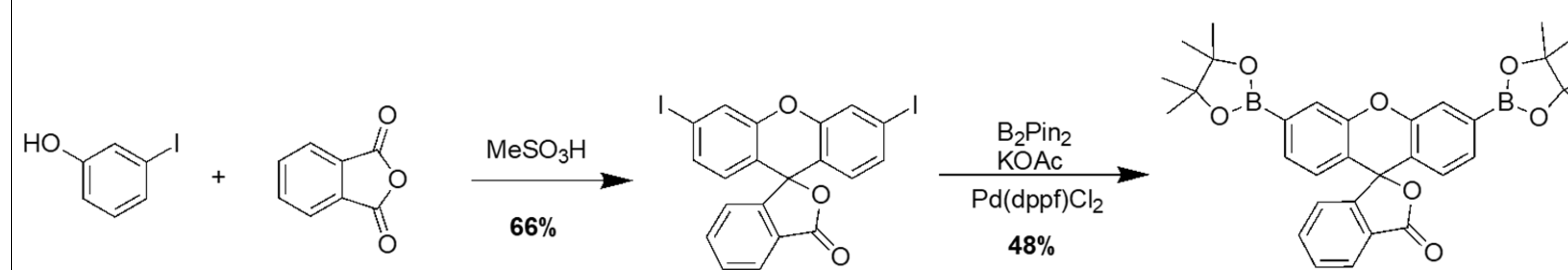


Figure 1. Synthesis of literature-reported PF1

Purification of PF1

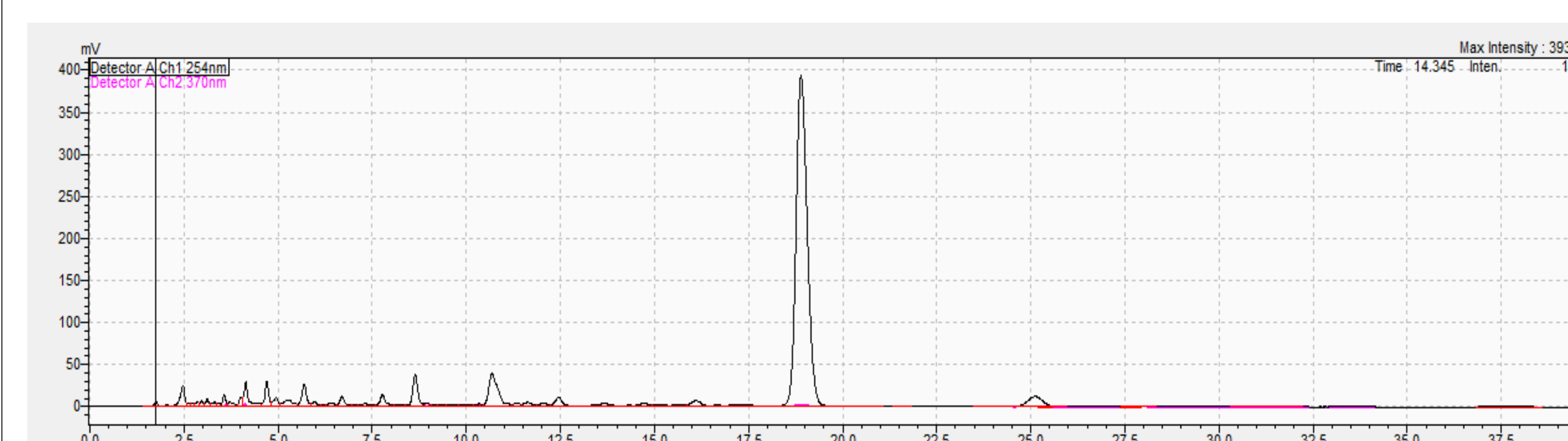


Figure 2. Chromatogram of PF1 coupled

Use of the HPLC will allow for final characterization and purification of the PF1 analog coupled with the azide handle before introduction into cells.

Synthesis of Clickable Analog

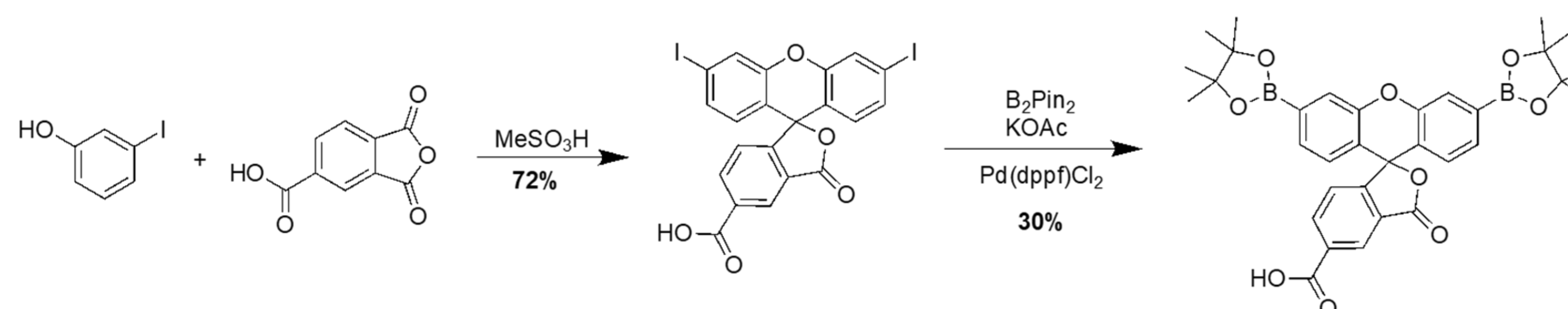


Figure 3. Synthesis of PF1 analog

NMR of Clickable Analog

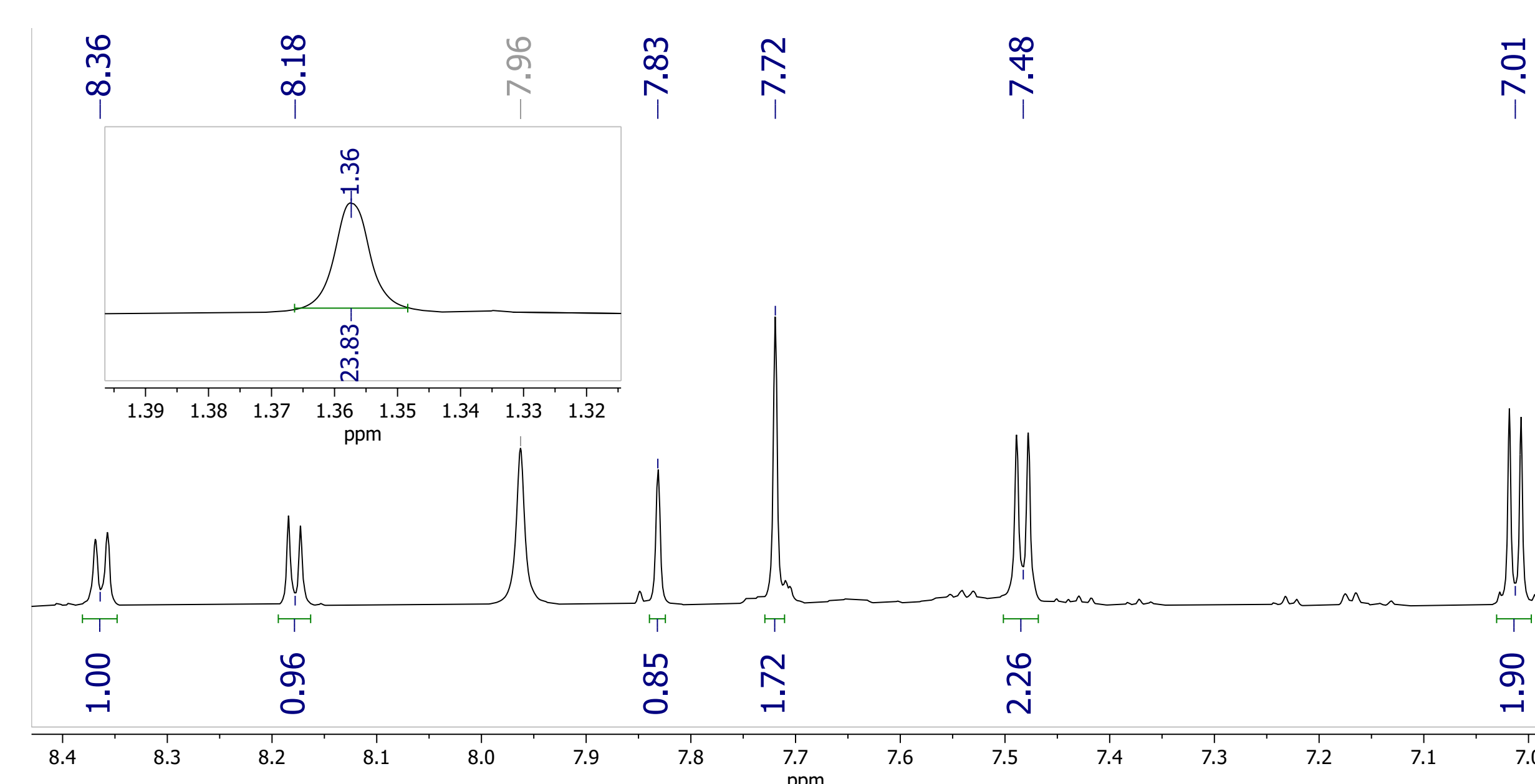


Figure 4. NMR of PF1 analog (ABS)

Future Work

- Use fluorescence spectroscopy to compare analyte selectivity of PF1 and the clickable probe

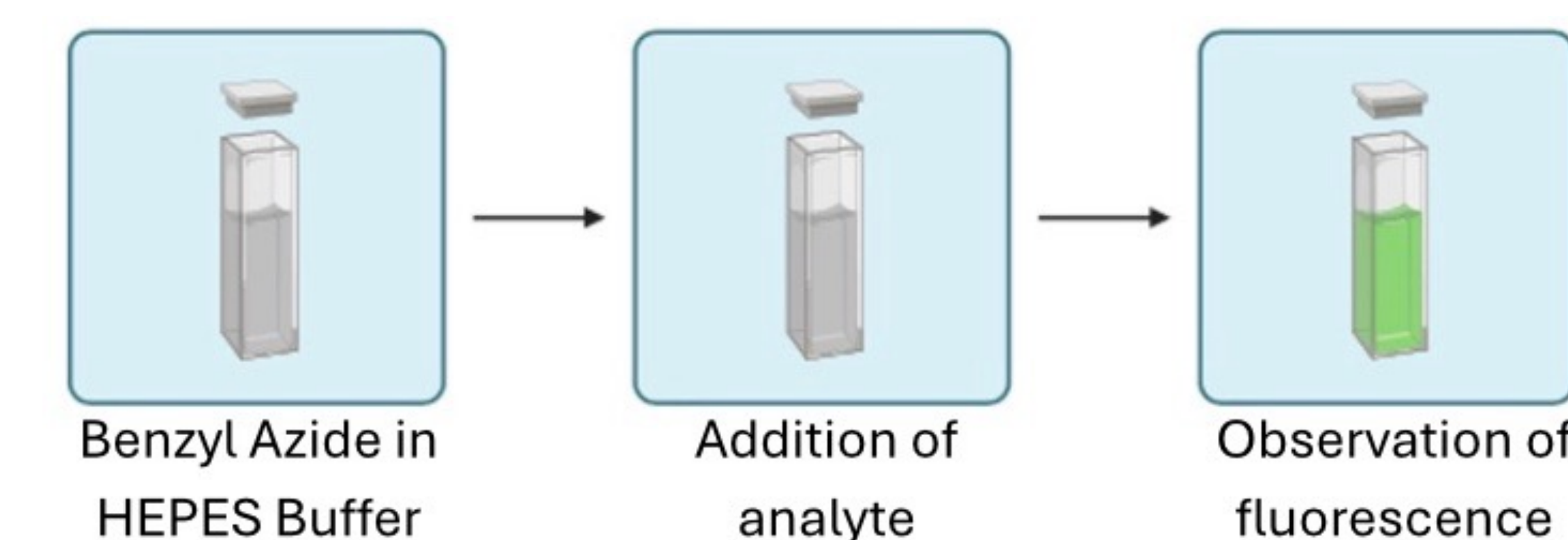


Figure 5. Procedure with the ABS reagent and observation of fluorescence with different analytes in an environment mimicking the cell membrane.²

- Image fluorescence response at membranes in the presence of hydrogen peroxide
- The images shown below demonstrate the fluorescence response of PF1 as reported in reference 3

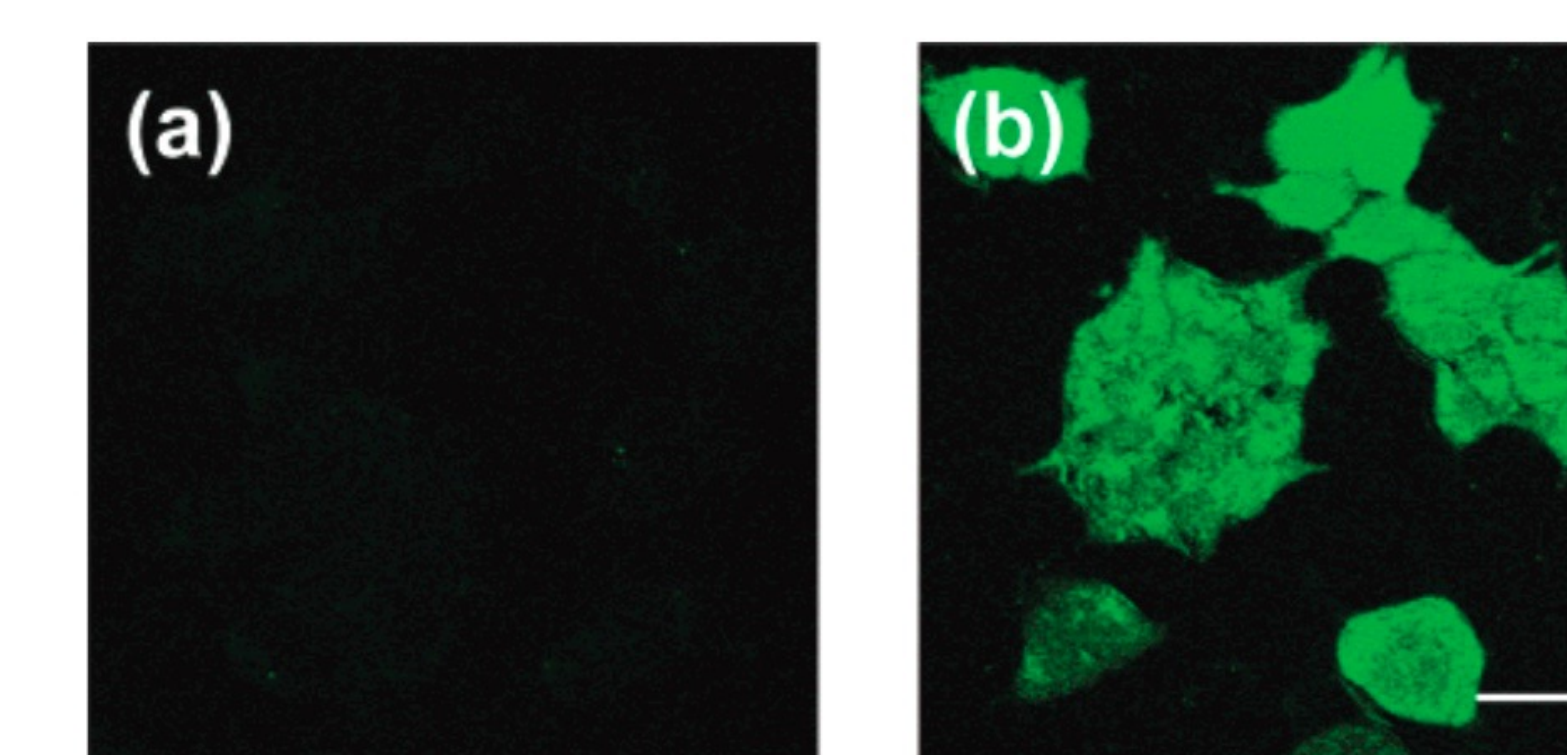


Figure 5. (a) Confocal fluorescence image of HEK cells incubated with PF1. (b) Confocal fluorescence image of HEK cells incubated with PF1 and treated with H₂O₂.³

References

1. Chang, M. C. Y.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. A Selective, Cell-Permeable Optical Probe for Hydrogen Peroxide in Living Cells. *J. Am. Chem. Soc.* **2004**, *126* (47), 15392–15393.
2. Created in <https://BioRender.com>
3. Miller, E.W.; Albers, A. E.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. Boronate-Based Fluorescent Probes for Imaging Cellular Hydrogen Peroxide. *J. Am. Chem. Soc.* **2005**, *127* (47), 16652–16659.

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